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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANT: O'Brien	§	ART UNIT: 1642
	§	
FILED: July 13, 2001	§	EXAMINER:
	§	Huff, Sheela J.
SERIAL NO.: 09/905,083	§	
	§	
FOR: Compositions and Methods for the Early Diagnosis of Ovarian Cancer	§	DOCKET:
	§	D6223CIP/C/D

The Assistant Commissioner of Patents
BOX NON-FEE AMENDMENT
Washington, DC 20231

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DECLARATION UNDER 37 C.F.R. § 1.132

Dear Sir:

I, Timothy J. O'Brien, do hereby state as follows:

I am the inventor of the above-referenced patent application. I have read U.S. patent application serial no. 09/905,083 and I am aware of the content of the Office Action, including all prior art cited against the '083 application.

An issue relating to the patentability of the claimed methods is the degree of enablement provided by Applicant's specification. The following data are presented as evidence of enablement commensurate with the scope of the claims:

Stratum Corneum Chymotrytic Enzyme (SCCE) Peptides As Target Epitopes For Human $CD8^+$ Cytotoxic T Cells (CTLs)

Peptides that possessed binding motifs for HLA class I molecules were tested directly for their ability to induce specific $CD8^+$ CTL responses from normal adult donors as described below.

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Dendritic cell (DC) Stimulation of $CD8^+$ CTL Responses

Monocyte-derived dendritic cells were generated from peripheral blood drawn from normal adult donors of the appropriate HLA type. Adherent monocytes were cultured in AIM-V (Gibco-BRL) supplemented with GM-CSF and IL-4 according to standard techniques (Santin et al, 2000). After 5-6 days, dendritic cell maturation was induced by addition of PGE_2, IL-1β and TNFα for a further 48 h.

Mature dendritic cells were loaded with peptide (2×10^6 dendritic cells with 50 µg/ml peptide in 1 ml serum-free AIM-V medium for 2 h at 37°C) and washed once prior to culture with 1×10^6/ml peripheral blood mononuclear cells (PBMC) in AIM-V or AIM-V plus 5% human AB serum. The PBMC:DC ratio was between 20:1 and 30:1. After 7 days, responder T cells were restimulated with peptide-loaded, irradiated autologous dendritic cells or PBMC at responder:stimulator ratios between 10:1 and 20:1 or 1:1 and 1:10 respectively. At this point, cultures were supplemented with recombinant human IL-2 (10-100 U/ml), and fed with 50-75% changes of fresh medium plus IL-2 every 2-4 days. T cell lines were established and maintained by peptide restimulation every 14-21 days. Responder $CD8^+$ T cells were purified by positive selection with anti-CD8-coupled magnetic beads (Dynal, Inc.) after the 2nd or 3rd antigen stimulation.

Peptide-Specific Cytotoxic Assay

Peptide-specific cytotoxicity was tested in standard 5-6 h microwell ^{51}Cr-release assays (Nazaruk et al, 1998). Autologous EBV-transformed lymphoblastoid cell

02/05/03 WED 17:11 FAX 5016868501 BIOTECH 003

lines (LCL) were loaded with peptide (50 μg/ml, 1 h at 37°C) and subsequently ^{51}Cr-labeled (50 μCi in 200-300 μl, 1 h at 37°C). Peptide-loaded ^{51}Cr-labeled LCL were incubated with $CD8^+$ T cells at effector-target ration between 10:1 and 1.25:1. Cytotoxicity was recorded as percentage ^{51}Cr released into culture supernatants.

SCCE PEPTIDE 5-13

SCCE peptide 5-13 (SEQ ID No. 33) is an HLA A2.1-binding peptide, as revealed by upregulation of A2.1 expression in T2 cells (data not shown). $CD8^+$ CTL specific for SCCE 5-13 killed peptide-loaded autologous LCL, but did not kill control, peptide-free LCL. Heterologous HLA A2.1-expressing peptide-loaded LCL were efficiently killed, but targets lacking HLA A2.1 were not killed (Figure 1).

SCCE PEPTIDE 123-131

SCCE peptide 123-131 (SEQ ID No. 32) is also an HLA A2.1-binding peptide, as revealed by upregulation of A2.1 expression in T2 cells (data not shown). $CD8^+$ CTL specific for SCCE 123-131 killed peptide-loaded autologous LCL, but did not kill control, peptide-free LCL. Heterologous HLA A2.1-expressing peptide-loaded LCL were efficiently killed, but targets lacking HLA A2.1 were not killed (Figure 2). Natural killer-sensitive K562 cells were not lysed. Cytotoxicity against SCCE 123-131 loaded LCL could be blocked with MAb specific for a non-polymorphic HLA class I determinant, confirming that lysis was HLA class I-restricted.

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CD8$^+$ CTL Specific for SCCE Peptide 123-131 Recognize Endogenously Expressed SCCE Tumor Antigen

To determine whether peptide-specific CD8$^+$ CTL are capable of recognizing targets that process and present endogenously expressed SCCE tumor antigens, recombinant adenoviruses expressing hepsin and SCCE, both in conjunction with green fluorescent protein (GFP) as a means of directly monitoring expression levels by flow cytometric techniques were constructed. It was found that CD8$^+$ CTL specific for SCCE 123-131 recognize and kill autologous targets infected with recombinant adenoviruses expressing the full-length SCCE antigen (Ad-GFP/SCCE) but did not recognize targets infected with Ad-GFP/hepsin (Figure 3). These results show that the SCCE 123-131 peptide is a naturally processed and presented CTL epitope for SCCE-specific CD8$^+$ CTL.

In conclusion, results disclosed above show that dendritic cells loaded with SCCE-derived peptides can efficiently stimulate HLA A2.1-restricted CD8$^+$ CTL responses in normal adults, demonstrating SCCE as a target antigen for immunotherapeutic purposes. Furthermore, SCCE 123-131 peptide is a naturally processed and presented CTL epitope for SCCE-specific CD8$^+$ CTL. Taken together, these data provide evidence that SCCE protein or fragments thereof are capable of inducing cytotoxic T cell responses against stratum corneum chymotrytic enzyme protein. Based on the data contained herein, I respectfully submit that the scope of the claims 22-31 in the '083 application has a reasonable correlation to the scope of the enablement provided.

005 BIOTECH 02/05/03 WED 17:12 FAX 5016868501

Figure Legend

Figure 1: $CD8^+$ CTL recognition of SCCE 5-13 peptide in a 5 h ^{51}Cr release assay. Targets are LCL loaded with SCCE 5-13 (•) and control LCL (O).

Figure 2: $CD8^+$ CTL recognition of SCCE 123-131 peptide in a 5 h ^{51}Cr release assay. Targets are LCL loaded with SCCE 123-131 (•) and control LCL (O).

Figure 3: Peptide-specific $CD8^+$ CTL recognition of endogenously processed and presented SCCE tumor antigen. CTL were derived by stimulation with dendritic cells pulsed with SCCE peptide 123-131. Cytotoxicity was tested in a standard 5 hours 51Cr-release assay against autologous macrophages infected with Ad-GFP/SCCE (■), macrophages infected with Ad-GFP-Hepsin (▲), macrophages pulsed with SCCE 123-131 peptide (■), or control untreated macrophages (●).

References

Nazaruk et al., Blood 91:3875-3883 (1998).

Santin et al., Obstetrics & Gynecology 96:422-430 (2000).

Santin et al., Am. J. Obstet. Gynecol. 183:601-609 (2000).

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001

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of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or patent issued thereon.

Date: 2/05/03

Dr. Timothy J. O'Brien

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Figure 1

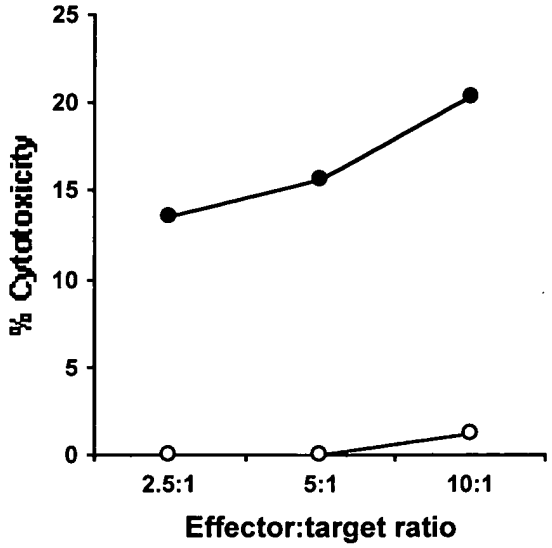

Figure 2

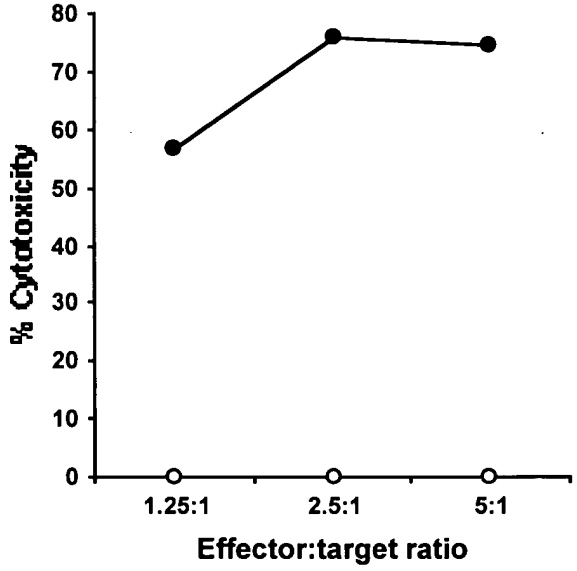

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Figure 3